Review article

Current insights into cellular senescence and myotoxicity induced by doxorubicin: The role of exercise and growth factors

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Abstract

Doxorubicin is an anti-neoplasmic drug that prevents DNA replication but induces senescence and cellular toxicity. Intensive research has focused on strategies to alleviate the doxorubicin-induced skeletal myotoxicity. The aim of the present review is to critically discuss the relevant scientific evidence about the role of exercise and growth factor administration and offer novel insights about newly developed-tools to combat the adverse drug reactions of doxorubicin treatment on skeletal muscle. In the first part, we discuss current data and mechanistic details on the impact of doxorubicin on skeletal myotoxicity. We next, review key aspects about the role of regular exercise and the impact of growth factors either administered pharmacologically or via genetic interventions. Future strategies such as combination of exercise and growth factor administration remain to be established to combat the pharmacologically-induced myotoxicity.

Key words: myotoxicity, doxorubicin, exercise, skeletal muscle, growth factors

Introduction

Doxorubicin is a chemotherapeutic drug widely used against a variety of malignancies such as acute leukaemia, non-Hodgkin lymphomas, Hodgkin's disease, breast cancer, lung cancer, childhood solid tumours and sarcomas [1, 2]. Doxorubicin is an antibiotic of the anthracyclines group used since the 1960s and it was the first approved liposomal injection in 1995 in the form of a hydrochloride salt of doxorubicin [3, 4]. Doxorubicin exhibits antineoplastic effects by inhibiting DNA replication to cause tumour cell death [5]. In brief, intercalation of doxorubicin with the DNA splits the double-strand and induces apoptosis by inhibiting macromolecular biosynthesis [6]. Inhibition of the topoisomerase II enzyme by doxorubicin, prevents DNA replication by DNA chain-breakage and prevention of DNA double-helix resealing [7, 8]. In addition, doxorubicin is oxidized to doxorubicin semiquinone (an unstable intermediate) and returns back to doxorubicin by producing mitochondrial reactive oxygen species (ROS). The increased ROS production causes oxidative stress that leads to cell death and apoptosis, and damages the cell membrane by lipid peroxidation [9]. Increased ROS enhances p53-DNA binding to activate the DNA cross-linking and caspase signalling and results in DNA damage and apoptosis [2]. However, the doxorubicin non-specific mechanism of action has deleterious effects on healthy cells and tissues that restrict its clinical use [1]. As a matter of fact, doxorubicin contributes to cachexia (i.e. a complicated metabolic syndrome related to underlying illness including malignancy and is presented by induced inflammatory process, insulin resistance, and increased protein turnover) and sarcopenia (i.e. loss of skeletal muscle mass and strength during ageing) in cancer patients due to increased chemotherapeutic toxicity on skeletal and cardiac muscles [2, 7, 10-12]. Cachexia is a significant death contributor in 20-30% of patients and 50% of patients suffer from it [13, 14]. In addition to cachexia, sarcopenia represents a comorbidity during cancer that affects the quality of life and increases the mortality rates of cancer patients [2, 7]. Among the main side effects of doxorubicin are nausea, hair and weight loss, fatigue, vasculature and liver toxicity, cardiotoxicity and at last but not least skeletal muscle atrophy [1, 2, 5, 12].

Herein, the aim of this review is to assimilate how doxorubicin causes muscle atrophy, senescence and toxicity and to discuss the current insights on the role of exercise and growth factors against doxorubicin-induced myotoxicity. The beneficial effects of the

exercise training on the skeletal muscle are well-established [15, 16]. Regular exercise promotes skeletal muscle functional adaptations including mitochondrial biogenesis, followed by increased antioxidant capacity [17, 18]. The alternations in muscle phenotype induced by exercise is responsible for the muscle protection against stress and more specifically, against doxorubicin-induced atrophy [18, 19]. The potential mechanisms of the protective effect of exercise against the doxorubicin-induced toxicity on skeletal muscle have been previously reviewed [18-20]. Furthermore, many studies focused on the regenerative effect of individual growth factors, such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) on various tissues and species [21-24]. These growth factors were mainly used as therapeutic strategies against various disorders such as senescence, wound healing, injury, muscular dystrophy, disease and chemotherapy. Finally, we suggest future directions for reversing the myotoxic effect of doxorubicin such as the combination of exercise and autologous biomaterials containing growth factors since their synergistic effects remain unexplored. For the implementation of the present review relevant studies were identified on PubMed by using combinations of the following key words: doxorubicin, myotoxicity, growth factors, exercise, cachexia and cancer complying with the ethical standards of the journal [25].

Doxorubicin induces skeletal muscle atrophy

Although skeletal muscle constitutes the most abundant organ of the human body with an outstanding regenerative capacity [12], doxorubicin treatment has numerous detrimental effects on skeletal muscle biology. It has been shown to affect muscle mass and size, ROS production, proteolysis via multiple pathways, autophagy, protein synthesis and disrupt the insulin pathway [5, 18]. The mechanism by which doxorubicin induces atrophy and toxicity in skeletal muscle is complex as it can directly and indirectly affect the muscle. Doxorubicin affects the skeletal muscle directly by interacting with it, and indirectly by inducing cardiotoxicity which subsequently impacts on the skeletal muscle [26]. For example, a common phenomenon in congestive heart failure is the reduced cardiac function which results in lower levels of blood flow towards skeletal muscle resulting in muscle dysfunction. The skeletal muscle dysfunction due to reduced cardiac function causes skeletal muscle wasting and weakness [26, 27]. Doxorubicin causes strength reduction, maximal twitch force

reduction and impairs skeletal muscle force generation against fatigue [4, 26, 28, 29]. Experimental evidence suggests that doxorubicin induces a profound mass loss in the extensor digitorum longus (EDL) muscle [4, 5]. In addition to skeletal muscle mass loss, muscle fibre cross sectional area (CSA), capillary density and the number of muscle satellite cells are significantly reduced [30, 31]. This effect is evident in type I, type IIa and type IIx/b muscle fibre CSA in the diaphragm, plantaris and soleus muscles [5]. The various pathways involved in doxorubicin-induced atrophy in skeletal muscle are illustrated in **Figure 1** and are discussed in the following sections. Key evidence from studies on doxorubicin administration impacting on skeletal muscle is presented in **Table 1.**

Doxorubicin induces oxidative Stress

It has been reported that doxorubicin increases ROS production which is responsible for increased oxidative stress and subsequent cell death [4, 26]. More specifically, doxorubicin decreases the mitochondrial respiratory capacity by inhibiting complex I- and II-supported respiration and by increasing H_2O_2 release, ending in reduced electron transport [5, 30, 32]. This effect on electron transport sets the basis of increased ROS [33]. In addition, the respiratory control ratio (a mitochondrial uncoupling and dysfunction indicator) is decreased in response to doxorubicin. Increased ROS production by mitochondria during doxorubicin administration causes oxidative damage to DNA and to protein [33]. Under the same mechanism, in the presence of doxorubicin, lipid peroxidation forms active aldehydes such as 4-hydroxy-2-nonenal (4-HNE, a lipid peroxidation biomarker) that forms adducts with muscle proteins to exacerbate oxidative damage [34]. In line with the augmented ROS, heat shock proteins (HSPs), which are important for protein synthesis and cell protection against oxidative stress, are reduced in response to doxorubicin [5]. Taken together, the disruption of mitochondrial respiration caused by doxorubicin leads to augmented synthesis of ROS, which in turn plays a key role in the induction of cell death and skeletal muscle atrophy (**Figure 1**).

Doxorubicin induces proteolysis and apoptosis and impairs protein synthesis

The ubiquitin-proteasome pathway is the main system for muscle protein degradation [11, 35]. During doxorubicin treatment, Forkhead-box (Fox) O1 and FoxO3 in muscle are elevated, which are directly associated with the activation of the E3 ligases, Atrogin-1 and MuRF-1, regulated by protein kinase B (Akt). E3 ligases regulate polyubiquitination, a key

step in the ubiquitin-proteasome system which is involved in skeletal muscle proteolysis by targeting proteins for degradation [11, 12, 35, 36]. Additional studies supported this data by revealing that doxorubicin inhibits Akt phosphorylation in muscle [4, 36]. Conversely, another study, failed to identify any alternations in genes related to induced proteolysis that lead to muscle atrophy during doxorubicin administration. However, when gene set enrichment analysis (GSEA) was done to identify minor gene changes, it was shown that FoxO1, ubiquitin-proteasome pathway and apoptosis were induced [14]. Furthermore, myostatin, a potent inhibitor of myogenesis, and upstream regulator of atrogin-1 and FoxO signalling, is increased in the skeletal muscle upon doxorubicin administration [35]. These data suggest that impairment of myogenesis and muscle repair due to increased proteolysis by upregulation of ubiquitin-proteasome pathway may induce muscle atrophy.

Furthermore, doxorubicin induces muscle atrophy by increasing the activity of calpain-1 and caspase-3 proteases [37, 38]. Calpain-1 and caspase-3 are enzymes that breakdown intact myofibrillar proteins and cleave structural proteins of the skeletal muscle to cause atrophy [37, 38]. Additionally, the doxorubicin-increased mitochondrial ROS formation induces proteolysis through oxidative alternations that augments myofibrillar protein (i.e. myosin, actin, troponin I, α-actinin) exposure towards calpain-1 and caspase-3 degradation [37, 38]. In line with this, myonuclear DNA damage by doxorubicin causes apoptosis via calpain-1 and caspase-3 activation, as indicated by the high number of TUNEL-positive nuclei (i.e. apoptosis marker) in skeletal muscle [4, 36, 39]. Apoptosis is induced by doxorubicin and skeletal muscle cellular damage, which is combined with muscle atrophy to cause a decrease of muscle cell number and muscle dysfunction [4]. Therefore, calpain-1 and caspase-3 upregulation by doxorubicin induces muscle protein degradation and leads to muscle cells apoptosis (**Figure 1**).

Doxorubicin has the potential to reduce protein synthesis through the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway, which is related to muscle size control. Evidence suggests that, during doxorubicin treatment, the phosphorylation of ERK1/2 is reduced but it returns to baseline after 2-4 weeks [4, 14]. Moreover, REDD1, a protein which has a role in muscle atrophy and is associated to reduced protein synthesis, is increased in response to doxorubicin [14]. It has been shown that REDD1 is induced through the p53-p21-REDD1 pathway, as it is activated by doxorubicin

[12]. Subsequently, protein synthesis is negatively affected by doxorubicin and possibly involved in muscle atrophy **(Figure 1)**.

Doxorubicin induces autophagy

Autophagy is a process characterized by the fusion of the autophagosome, a closed double-membrane vesicle containing a part of cytoplasm, with the lysosome, to degrade damaged organelles and protein aggregates to preserve the healthy function of cells [11]. However, it is believed that induced autophagy may result in cell death via apoptosis [5]. During doxorubicin administration, autophagy markers including Beclin-1 mRNA and protein, Atg12 mRNA and protein, Atg12-Atg5 protein, Atg7 protein and LC3 mRNA, which are essentials for the autophagosome formation and maturation, are significantly increased [36]. Moreover, LC3 II-to-LC3 I ratio, which indicates the formation of autophagosomes, is elevated [36]. Augmented autophagy is reported as early as 1 day following the doxorubicin administration without any changes 5 days post-administration [4]. Furthermore, cathepsin L is augmented, whereas cathepsin B and D shows no response to doxorubicin treatment. Cathepsin B, D and L are proteases found in lysosomal and their abundance is very high during muscle atrophy [36]. Additionally, following doxorubicin administration, BCL2/adenovirus E1B 19kDa interacting protein 3 (BNIP3) found in skeletal muscle was increased, which is an autophagy activation protein that also induces apoptosis and mitochondrial dysfunction [35]. Taken together, it appears that elevated activation of autophagy occurs at 24 hours after doxorubicin treatment, it is normalized 5 days post-doxorubicin administration and it may induce skeletal muscle atrophy [4, 35, 36]. The molecular events of doxorubicin-induced autophagy that lead to muscle atrophy are presented in **Figure 1**.

Doxorubicin disrupts insulin signalling

Evidence suggests that doxorubicin chemotherapy can indirectly cause muscle atrophy through glucose intolerance. High glucose, free fatty acids and insulin levels have been detected in plasma, three days post-doxorubicin administration [2]. Even though increased insulin resistance has been detected in response to doxorubicin treatment, the insulin-like growth factor (IGF) 1 receptor, the phosphoinositide 3-kinase (PI3-K) and the Akt protein expression in skeletal muscle remained unaltered. Despite that, proteins of the insulin pathway such as insulin receptor substrate 1 (IRS-1) and glycogen synthase kinase 3 beta

(GSK3-B), and protein and mRNA levels of the glucose transporter type 4 (GLUT4) and the AMP-activated protein kinase-alpha (-α) were reduced [2]. However, the AMPK modulation by doxorubicin remains controversial, as other studies showed that doxorubicin induces AMPK activation and is related to the increased cell death, apoptosis and ROS production [40, 41]. According to these studies, muscle atrophy due to doxorubicin can affect the insulin signalling pathway which in turn further induces muscle atrophy, as protein synthesis is reduced due to impaired expression of proteins related to glucose uptake [2, 5, 42]. Therefore, the doxorubicin induced muscle atrophy disrupts the insulin signalling pathway, which in turn mediates the disruption back and induces muscle atrophy (**Figure 1**).

Doxorubicin induces cellular senescence

Several studies (see **Table 2**) have shown that doxorubicin induces cellular senescence in various cells types including skeletal muscle cells, embryonic ventricular myocardial cells, endothelial progenitor cells (EPCs) and vascular smooth muscle cells (VSMCs) [40, 41, 43, 44]. Some studies showed that doxorubicin induces the activation of the AMPK which leads to increased cell death and apoptosis through: the increased ROS production that damages cell DNA; the increased activation of p53 (cell death and apoptosis regulator) and JNK (apoptosis marker); and the inhibition of mammalian target of Rapamycin (mTORC)1 [40, 41]. However, increased mTOR signalling may induce senescence through reduced autophagy by the increased activation of the senescence markers p53/p21/p16. p53/p21/p16 act as tumour suppressors inducing senescence through cell cycle arrest [44]. Furthermore, increased expression of the transcription factor E2F1 has been reported in p16-defective cells showing that apoptosis due to p16 is mediated through the E2F1. Activation of E2F1 is related to cell proliferation as an oncogene or to cell death as a tumour suppressor. p16, as a tumour suppressor, has the ability to modulate the E2F1 by negative control of the mRNA decay-promoting AUF1 protein. Also increased expression of the E2F1 was seen in cells ectopically expressing p16, representing that p16 sensitizes the cells to doxorubicin through E2F1 [45]. In addition, Spallarossa et al. reported increased activity of p16 and JNK, and reduction in proliferation and cell viability [43]. Also, telomeric repeat-binding factor 2 (TRF2), a protein responsible for preserving the t-loop telomeric structure that governs chromosomal stability, was reduced. This leads to senescence by telomere shortening and dysfunction [43]. Moreover, increased miR-375 expression reduced the

proliferation of K562 cells. An inversely proportional relationship was observed between miR-375 against 14-3-3zeta (anti-apoptotic gene) and SP1 genes (transcriptional regulator) which are related to cancer development and progression [46]. Reduced miR-375 expression leads in an upregulation of 14-3-3zeta and SP1 and promotes a survival effect for cancer cells. On the other hand, increased miR-375 and downregulated 14-3-3zeta and SP1 induces cellular senescence. Increased autophagic genes Atg9B and Atg18, during doxorubicin administration, indicated increased activation of autophagy in the doxorubicin-induced senescence cells [46]. Concludingly, doxorubicin induces skeletal muscle senescence through an upregulation of apoptotic and senescence markers such as JNK, p16 and p53 and reduction of anti-apoptotic markers and telomere preserving proteins such as 14-3-3zeta, SP1 genes and TRF2. Augmented proteolysis due to oxidative stress, autophagy and ubiquitin-proteasome pathway activation, in line with decreased protein synthesis due to changes in response of growth-promoting pathways, can lead to muscle atrophy and cellular senescence. Therefore, strategies including exercise and growth factor administration as potential tools against the doxorubicin-induced muscle atrophy and toxicity are critically discussed in the following section.

The Effect of Exercise on Doxorubicin-Induced Myotoxicity

In an attempt to gain mechanistic insights of how exercise training prevents the doxorubicin-induced atrophy on the skeletal muscle, recent studies (see **Table 3**) have focused on the soleus and EDL muscles of rats exposed to endurance exercise using a treadmill [36, 47, 48]. It was previously believed that exercise-induced ROS production would aggravate the toxicity of doxorubicin however, this is not supported by data illustrating the therapeutic effects of exercise against doxorubicin toxicity [20, 49].

Bredahl et al. examined the effects of resistance and endurance training on the soleus and EDL muscle of rats against doxorubicin treatment [50]. Resistance training was achieved by a model of chronic hind limb loading while endurance training was performed on a treadmill at various speeds, inclines and durations. It was found that the maximal twitch force and the maximal rate of force decline were maintained in the soleus muscle of the doxorubicin resistance training group compared to the doxorubicin sedentary group. The doxorubicin-induced fatigue was reduced in the soleus, but not EDL, in the endurance training group compared to the sedentary group [50]. Bredahl et al., in a more recent study, investigated

the effects of resistance training combined with creatine monohydrate administration on the soleus and EDL muscle of rats against doxorubicin-induced myotoxicity [48]. The same model of chronic hind limb loading was used and creatine was administrated after the muscles were isolated. They have shown that doxorubicin-induced fatigue was delayed by 20s in the soleus and 10s in the EDL post resistance training compared to the sedentary group. In addition, the doxorubicin-induced fatigue was delayed by 50s in the soleus and 20s in the EDL when resistance training and creatine treatment were combined [48]. De Lima et al. studied the properties of endurance exercise on the murine gastrocnemius against the deleterious effects induced by the doxorubicin administration [42]. Endurance exercise was performed at various speeds on a treadmill and was referred as aerobic exercise. They have shown that exercise increases the maximal aerobic capacity of the mice treated with doxorubicin and mitigates the negative effect of doxorubicin on protein synthesis and the doxorubicin-induced fatigue compared to the control group treated with doxorubicin. Furthermore, it was found that exercise activates AMPK which is reduced by doxorubicin administration [42]. AMPK has a significant role in cellular metabolism regulation and when inhibited by doxorubicin impairs glucose uptake [2, 42]. Another study reported that endurance exercise prevented the doxorubicin-induced REDD1 protein on the rat soleus muscle [51]. REDD1 negatively affects the muscle size by inhibiting mTOR signalling, compared to sedentary animals treated with doxorubicin. Moreover, it was found that exercise maintained the MHC I fiber size and the phosphorylation of the mTORC1 and its related 4E-BP1 protein which regulate muscle protein synthesis. The LC3BII/I ratio was also maintained in the exercise group compared to the sedentary group which is related with slower mitochondrial turnover due to the maintained rate of LC3 lipidation which eventually may result in ROS formation [51]. Guigni et al. examined the effect of exercise using an in vitro model of contraction and mechanotransduction by electrical stimulation in C2C12 myotubes treated with doxorubicin [52]. It was shown that electrical stimulation prevents doxorubicin-induced myotube myosin content loss and increased Murf1, an E3 ligase related to muscle proteolysis compared to non-electrical stimulated cells. Additionally, the in vitro model of exercise preserved the mitochondria content and the phosphorylation of Akt and FoxO3a (Akt is activated during muscle contraction which then phosphorylates the FoxO3a) [52]. Huang et al. accessed the effects of eccentric exercise on rat soleus muscle administered with doxorubicin [7]. The eccentric exercise protocol was an acute bout of

decline treadmill running. The exercise prevented the increased inflammation score and increased M1 macrophage, which is involved in the phagocytic events during early phase of inflammation, in the doxorubicin-treated exercised rats compared to the sedentary. M2 macrophage which is involved in the regenerative phase of inflammation was increased in the exercised group. Moreover, the amount of necrotic and centrally nucleated fibres was decreased [7]. Quinn et al. investigated the effects of endurance exercise (i.e. treadmill running) and doxorubicin treatment on the myogenic regulatory factors using the soleus, EDL and diaphragm muscles of rat [47]. They found that exercise augments the myogenic regulatory factor Myf5 in soleus and diaphragm muscles and MyoD & Mrf4 in soleus muscle compared to sedentary group treated with doxorubicin [47]. Yoon et al. studied the effects of endurance exercise on murine C2C12 cells [41]. The addition of the pharmacological AMPK agonist, 5-aminoimidazole-4- carboxamide-1-b-D-ribofuranoside (AICAR) to the cells represented an in vitro exercise mimetic model as AICAR shows similar effects to exercise including reduction of fat mass, augmentation of oxygen consumption and improvement of endurance capacity. They found that AICAR decreased cell apoptosis and increased cell viability and activation of AMPK [41]. Kavazis et al. examined the effects of short-term endurance exercise on acute doxorubicin-induced FoxO transcription in cardiac and skeletal muscle of rats [35]. Short-term endurance was performed on a treadmill by increasing the duration of the training every day for five days followed by two days of rest for an overall duration of two weeks. Exercise prevented the doxorubicin-induced increase of FoxO1 and Murf1 in cardiac muscle and the increase of FoxO3, Murf1 and BNIP3 in soleus muscle compared to the sedentary group treated with doxorubicin. Activated FoxO signaling is induced by increased ROS formation caused by doxorubicin. Upregulation of FoxO signaling leads to an increased expression of FoxO target genes including Murf1 and BNIP3 that are associated with muscle degradation and atrophy [35]. In addition, exercise increases peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α) which promotes mitochondria biogenesis and can inhibit FoxO transcriptional activity, thus it can protect muscle from doxorubicin-induced atrophy [35]. Smuder et al. accessed the effects of endurance exercise on doxorubicin-induced markers of autophagy signaling in the soleus muscle of rats [36]. This study found that exercise prevents the doxorubicin-induced damaged myofiber ultrastructure and cell apoptosis compared to the sedentary group administered with doxorubicin. Moreover, autophagic regulators involved in

autophagosome formation and maturation such as Beclin-1, Atg12 mRNA and protein, Atg7 protein and LC3 mRNA or the LC3 II-to-LC3 I ratio, a marker of autophagosomes formation, which were induced in the control group, were prevented by the endurance exercise [36]. In another study, Smuder et al. studied the effects of endurance exercise on doxorubicin-induced oxidative stress and proteolysis in the soleus muscle of rats [38]. Exercise protected muscle from preventing doxorubicin-induced proteins carbonyls and 4-HNE which increase oxidative damage. Moreover, degradation of actin and proteolysis were prevented as the calpain and caspase-3 activity, which are responsible for this damage and are related to muscle atrophy, were prevented by exercise [38]. The HSP72 and the GPX1 proteins which are responsible protein synthesis and protection against oxidative stress were upregulated by exercise [38]. Kwon examined the effects of endurance exercise on skeletal muscle remodelling against doxorubicin-induced myotoxicity in murine soleus muscle [53]. The protective effects of exercise originated in the prevention of doxorubicin to induce irregular myofiber size and central nucleation and a fibre type I transition favourable for oxidative metabolism. Exercise restored the FoxO3α to basal levels as it was activated by doxorubicin and restored the expression of α-ACTN, a structural protein of the Z-line inhibited by doxorubicin [53]. Taken together, many forms of exercise such as endurance, resistance and eccentric protect the skeletal muscle from the doxorubicin-induced atrophy via many pathways.

The Effect of Growth Factors Against Doxorubicin-Induced Myotoxicity

A different approach than physical exercise to reverse doxorubicin-induced toxicity has been the administration of growth factors. The potent mitogenic, angiogenic and migration properties of growth factors that are essential in tissue regeneration are well documented. In fact, therapeutic effects of various growth factors such as PDGF, EGF, VEGF, FGF and HGF against doxorubicin-induced myotoxicity have been reported [21-24]. In the next sections we discuss the findings of genetic and pharmacological studies using growth factors against doxorubicin toxicity (see **Table 4**).

Pharmacological Administration of Growth Factors in Doxorubicin-Treated Cells

Among other studies, growth factors were used against doxorubicin-induced myotoxicity and wound healing. Lawrence et al. tested the effect of transforming growth factor beta (TGF-β), EGF and PDGF, individually and synergistically, on wound chamber models

extracted from doxorubicin-treated rats by incubating them with 100 ng/ml of each growth factor. It was found that TGF-β accelerates wound healing and a combination of TGF-β, EGF and PDGF was able to completely reverse the impairment of wound repair caused by doxorubicin [21]. Koleini et al. demonstrated that FGF-2 has the capacity to protect cardiomyocytes from the cardiotoxic effects of doxorubicin via the mTOR/Nrf-2/HO-1 pathway, by incubating rat cardiomyocytes with 10 ng/ml of FGF-2 [24]. FGF-2 decreased the lactate dehydrogenase (LDH) activity (i.e. an indicator of disruption of cardiomyocyte plasma membrane integrity) and reduced ROS production and the pro-apoptotic markers such as p53, caspase-3 and BNIP3 [24]. In addition, FGF-2 reversed cell death and mitochondrial permeability transition pores (mPTP) formation caused by doxorubicin. FGF-2 increased the mRNA and protein expressions of Nrf-2 and HO-1 which are endogenous cytoprotective antioxidant regulators, and induced the mTOR activity, which controls cell growth and inhibits the initiation of autophagy [24]. Koleini et al. have also demonstrated an experiment using non-mitogenic FGF-2 against doxorubicin-induced cardiomyocyte toxicity [54]. By incubating rat cardiomyocytes with 10 ng/ml non-mitogenic FGF-2 they were able to identify a protective effect against doxorubicin toxicity. Non-mitogenic FGF-2 was able to prevent augmentation of ROS and upregulation of fragmented and non-fragmented oxidized phosphatidylcholine species. Moreover, cardiomyocyte damage and cell death were reduced whereas the phosphorylation of ERK (cardiac pro-survival kinase) was increased. Finally, they showed that the protective effect of non-mitogenic FGF2 is mediated through the FGFR1/ERK signalling [54]. Sontag et al. demonstrated the effect of 10 μg of either FGF2 or FGF16 on murine heart under doxorubicin conditions. Both FGFs had the same protecting properties of mitigating the doxorubicin-induced poisoning effect in the left ventricular developed pressure [55]. Wang et al. used 50 ng/ml of FGF21 on rat cardiomyocytes in vitro and 100 ng/ml FGF21 on mice heart in vivo to test any regenerative properties against doxorubicin induced toxicity [56]. They showed that FGF21 induces the activation of Sirt1/liver kinase B1 (LKB1)/AMPK pathway which through this activation, doxorubicin-induced toxicity is prevented, as inflammation in heart, apoptosis and oxidative stress are suppressed. On the other hand, inflammatory cytokines related to heart dysfunction such as tumour necrosis factor (TNF-α) and IL6, and cell death related ROS production and Bax/Bcl-2 expression, were all decreased [56]. Yao et al. used either 500 or 20 ng/ml of EGF to investigate any properties against doxorubicin toxicity on various cell lines such as murine

C2C12, human embryonic kidney 293 cells and lung adenocarcinoma epithelial A549 cells [57]. It was found that cell viability was increased by the EGF as doxorubicin-mediated growth arrest was diminished by the promotion of the cell cycle-associated protein cyclin D1, which induces proliferation. The induced GATA Binding Protein 4 (GATA4) expression contributed to this augmented cell survival, as it promotes the cyclin D1 expression [57]. Chen et al. proved that mesenchymal stem cells (MSCs) induced the release of VEGF against the doxorubicin-induced cellular senescence on cardiomyocytes and was able to rescue the affected cells [58]. This MSCs-induced VEGF release increased the cell viability and the proliferation, decreased the p53 and p16 expression, and reduced the telomere shortening and telomerase activity compared to the control [58]. Therefore, the augmented presence of growth factors such as PDGF, EGF, FGF and VEGF either by cell incubation or boosting via a mediator, increased the wound healing process and presented protective effects on the heart against doxorubicin-induced injury and toxicity.

Gene therapy delivering growth factors in doxorubicin-treated cells

Gene therapy using growth factors has been used as an alternative procedure for the pharmacological induction. Adenovirus (Adv) transfection was used to induce the experimental cells with the desired growth factor. Räsänen et al. used VEGF-B gene therapy to prevent doxorubicin-induced cardiotoxicity [23]. VEGF-B gene therapy was found to prevent the decrease of heart weight, cardiomyocyte size, left ventricle posterior wall, septum thickness, body mass, coronary capillary area and ERK1/2 phosphorylation caused by doxorubicin administration [23]. Furthermore VEGF-B prevented microvasculature cardiac damage and protected from apoptosis and endothelial dysfunction induced by doxorubicin. Cytoskeleton biogenesis, angiogenesis, cell cycle-related transcripts, left ventricle mass and systolic and diastolic volumes and mitochondrial DNA content where increased, while DNA damage induced by doxorubicin was reduced by VEGF-B gene therapy [23]. Chen et al. examined the effect of VEGF165 expression by Adv delivery on rat cardiomyocytes against doxorubicin administration [59]. According to their outcome, VEGF165 increased the Bcl-2 protein and induced the Akt/nF-kB/Bcl-2 signalling pathway. Chen et al. reported that Bcl2 is an anti-apoptotic factor as it prevents the release of cytochrome c, which can activate apoptotic factors such as caspase-9. The effects of VEGF165 on cell survival may have been brought about by the Akt/nF-kB/Bcl-2 signalling

pathway. Caspase-3 and Fas-Associated protein with Death Domain (FADD)/caspase-8 which are apoptotic markers, were reduced by the VEGF165 [59]. FGF-16 has revealed protective effect on heart and cardiomyocytes against doxorubicin administration [60]. It was shown that FGF-16 increased the resistance to doxorubicin-induced cardiomyocyte damage and decreased the LDH activity as well as the apoptotic marker, annexin-V^+ cells [60]. Esaki et al. investigated the effect of Adv HGF delivery on mouse cardiac muscle and cardiomyocytes [22]. HGF reduced the left ventricular dilatation and dysfunction of heart, the cardiomyocyte atrophy and the myocardial fibrosis. It was also found that HGF induced the expression of GATA4 and MHC. GATA4 protein is related with antiatrophic effects on heart as it promotes cardiac growth [22]. The activation of ERK and the c-Met/HGF receptor were induced by the HGF. The ERK/MAPK pathway which is related to heart hypertrophy, is activated through c-Met/HGF receptor signalling [22]. Overall, delivery of growth factors by cell transfection is protective against doxorubicin chemotherapy and may reverse atrophy and boost cardiac myogenesis.

Conclusion

In conclusion, many of the articles cited herein have contributed to our understanding of how doxorubicin's mechanism of action induces myotoxicity. Furthermore, other articles cited in this review have expanded our knowledge in terms of how exercise and growth factors are individually used against doxorubicin-induced myotoxicity to prevent or reverse this effect. However, as cancer mortality has reduced in the recent years, the patients suffering from the long-term effects of myotoxic antineoplastic drugs are continuously increasing. Thus, future strategies such as combination of exercise and growth factor administration remain to be established to combat the doxorubicin-induced myotoxicity.

Future directions

Platelet releasate collected from aggregated platelets after the removal of cellular debris, contains multiple growth factors such as endothelial cell growth factor (ECGF), IGF, TGF-b, PDGF, VEGF, EGF and FGF known to enhance proliferation, migration and angiogenesis [61, 62]. Accumulating evidence suggests that platelet releasate can be used as an autologous biomaterial to promote skeletal myogenesis and accelerate skeletal muscle regeneration after acute injury, reduce markers of inflammation and apoptosis and promote myocellular proliferation [61, 63-66]. We have recently shown that platelet releasate regulates skeletal

myogenesis and muscle stem cell fate in a dose-dependent manner via the PDGF/ VEGF-Cyclin D1-MyoD-Scrib-Myogenin axis and maintains the ability of satellite cell to differentiate [64, 65, 67]. However, the role of platelet releasate in myogenesis after doxorubicin administration remains undetermined. Therefore, future research is needed to determine the muscle regenerative properties of platelet releasate post-doxorubicin treatment. Furthermore, the synergistic effects of the exercise and growth factors have not experimentally addressed yet. Thus, the combination of the two strategies against doxorubicin-induced myotoxicity remains to be examined in future work to identify any synergistic effects.

Figure legend

Figure 1: Doxorubicin affects multiple signalling pathways that induce muscle atrophy and senescence. Doxorubicin interferes with the DNA by intercalation and inhibits the topoisomerase II enzyme, therefore DNA chain breaks and DNA replication and transcription are prevented. Oxidation of doxorubicin to doxorubicin semiquinone and back, produces mitochondrial reactive oxygen species (ROS). The increased ROS production causes cell death via oxidative stress and DNA damage by p53-DNA binding. Increased ROS damages the cell membrane by lipid peroxidation. In the red pathway: mitochondrial degradation is increased due to augmented reactive oxygen species (ROS) production. Increased ROS activates calpain-1 and caspase-3 which results in proteolysis and eventually muscle atrophy (green pathway). In addition, increased ROS induces mitochondrial degradation and subsequent muscle atrophy. In the green pathway: protein degradation is increased as a result of calpain-caspase and ubiquitin-proteasome proteolysis and induced autophagy. As myostatin (Mstn) is increased, forkhead (FOXO) family transcription factors are activated and in turn upregulate atrogin-1 and MuRF-1. In the blue pathway: protein synthesis is reduced as a result of disrupted insulin pathway. Insulin-like growth factor 1 (IGF-1) enhances protein synthesis via Akt and mTOR however doxorubicin disrupts this pathway as well as glucose transporter type 4 (GLUT4) and AMP-activated protein kinase (AMPK) involved in glucose uptake, which ends in decreased protein synthesis. Finally, in the yellow pathway: protein synthesis is decreased due to increased REDD1 through activation of p53/p21 pathway by doxorubicin.

Tables

Table 1: Characteristics and outcomes from doxorubicin studies against skeletal muscle

Reference	Species	Origin skeletal muscle sample	Dose (mg/kg)	Number of doses	Doxorubicin effect on skeletal muscle atrophy
de Lima Junior et al. 2016[2]	Wistar rats	EDL	15	1	↓: Muscle Weight & CSA; testosterone levels; AMPk; glucose uptake; IL-6/TNF-α ↑: Corticosterone levels; systemic insulin resistance
Yu et al. 2014[4]	C57BL/6J mice	Gastrocnemius	15	1	↑: Myofibres with centralized nuclei; TUNEL apoptotic index; cell death; Bax & Bcl-2 proteins; LC3II-to-LC3I ratio ↓: p-Akt/total Akt & p-ERK/total ERK proteins
Hulmi et al. 2018[12]	C57BL/6J mice	TA, gastrocnemius, soleus	Long- term exp: 6 Acute exp: 15	Long-term: 4 Acute: 1	↑: p21/Cdkn1a; Atrogin1 mRNA; p53 protein; Myod1 mRNA; Redd1/Ddit4 ↓: Tfrc mRNA; Pgc-1α exon 1a; Pgc-1α exon 1c; Pgc-1β; Activin A; Gdf11 mRNA
Nissinen et al. 2016[14]	C57BL/6J mice	TA, gastrocnemius, soleus	Long term exp. 1-3:6 Acute exp. 4:15 Long term exp. 5-6:12	Exp. 1-3:4 Exp.4: 1 Exp. 5:2	↓: Muscle weight & CSA; lean mass & fat mass; bone mineral density & bone mineral content; maximal running performance; Ulk1 & Becn1 genes; apoptosis; protein synthesis; phosphorylation of ERK ½; blood haemoglobin & haematocrit ↑: FOXO1; REDD1
Gilliam et al. 2016[30]	C57BL/6N mice	Soleus	20	1	↓: Muscle weight & CSA; body weight; lean mass & fat mass; ability to scavenge H_2O_2; complex I- and complex II-supported respiration; maximal isometric tetanic force ↑: Mitochondrial H_2O_2-emitting potential: global protein carbonylation; oxidative modifications of myofibrillar proteins
Gilliam et al. 2013[33]	Sprague Dawley rats	Gastrocnemius	20	1	↓: Body weight; lean mass & fat mass; body oxygen consumption; ambulatory activity; total energy expenditure; respiratory exchange ratios; NADH-supported respiration; FADH2-supported respiration; complex I- and complex II-supported respiration ↑: Mitochondrial H_2O_2-emitting potential; potential for electron leak; potential for ROS production
Hydock et al. 2011[27]	Sprague Dawley rats	Soleus & EDL	dose 1: 10 dose 2: 12.5 dose 3: 15	1	↓: Body mass in dose 1 & 2; maximal twitch force; maximal rate of force production; rate of force decline ↑: Muscle fatigue
Kavazis et al. 2014[35]	Sprague Dawley rats	Soleus	20	1	↑: FoxO1; FoxO3; Atrogin-1/MaFbx; MuRF-1 & BNIP3 mRNA in sedentary; myostatin mRNA in sedentary ↓: pAMPK/AMPK

Table 1: Characteristics and outcomes from doxorubicin studies against skeletal muscle

Reference	Species	Origin skeletal muscle sample	Dose (mg/kg)	Number of doses	Doxorubicin effect on skeletal muscle atrophy
Smuder et al. 2011[38]	Sprague Dawley rats	Soleus	20	1	↑: Carbonyl derivatives in myofibrillar protein; 4-HNE protein conjugates; CuZn-SOD; Mn-SOD; catalase; calpain; caspase-3; α-II spectrin calpain-specific cleavage; α-II spectrin caspase3-specific cleavage; easily releasable myofilaments ↓: GPX1; HSP72; actin
Smuder et al. 2011[36]	Sprague Dawley rats	Soleus	20	1	↑: Muscle Damage; TUNEL-positive nuclei; Beclin-1; Atg12 mRNA & protein; Atg12-Atg5 complex; Atg7 proteins; LC3 mRNA; LC3II to LC3I ratio; cathepsin L mRNA & protein
Bredahl et al. 2017[26]	Sprague-Dawley rats	Soleus & EDL	Incubated in 24 μM	2 incubations	↓: Rate of force production; rate of force decline ↑: Muscle fatigue
Gibson et al. 2014[1]	Sprague Dawley rats	Soleus & EDL	15	1	↑: Muscle fatigue as animal aged from 4 to 24 weeks; DOX accumulation in EDL as animal aged from 4 to 24 weeks ↓: MRP-2 & MRP-7 in EDL as animal aged from 4 to 24 weeks
Hayward et al. 2012[29]	Sprague Dawley rats	Soleus & EDL	15	1	↓: Maximal twitch force; rate of force development; rate of force decline
Sin et al. 2015[39]	SAMP8 mice	Gastrocnemius	18	1	↓: Body Mass & Muscle Mass; SIRT1 in old, deacetylase activity of SIRT1; PDK1 protein; phosphorylation of $mTOR^{Ser2481}$; phospho-Akt^{Ser473} in young ↑: PDK4 in young; Bax protein expression; caspase 3 activity & apoptotic DNA fragmentation; MuRF-1; ubiquitinated proteins; proteasomal activity
Gouspillou et al. 2015[32]	C57BL/6 mice	Gastrocnemius, plantaris & quadricep	10	Early group: 2 Late group: 4	↓: Body Mass & Muscle Mass; fiber size; complex I- and complex II-supported respiration; parkin protein; parkin/VDAC ↑: Mitochondrial ROS production
D'Lugos et al. 2019[31]	Sprague Dawley rats	Soleus & EDL	4	3	↓: Muscle fiber siz; Pax7-positive satellite cells; capillary content in soleus; MGF mRNA in EDL ↑: MYF5 mRNA in soleus
de Lima et al. 2018[42]	C57BL/6 mice	Gastrocnemius	2.5	12	↓: Body weight; fat & lean body mass; muscle weight; physical capacity; CSA; protein synthesis ↑; Basal glycemia; corticosterone

Table 2: Characteristics and outcomes from doxorubicin studies inducing cellular senescence

Reference	Species	Dose (μM)	Doxorubicin effect
Yoon et al. 2019[41]	C2C12 skeletal myoblast cells	1	↑: AMPk phosphorylation; cell death; β-galactosidase
Chen et al. 2011[40]	Rat embryonic ventricular myocardial H9c2 cells	0.17, 0.52, 0.85, & 1.71	↑: AMPkα phosphorylation; cell death and apoptosis; ROS-dependent LKB1 activation; JNK activation; mTORC1 inhibition; p53 activation
Al-Khalaf et al. 2011[45]	U2OS, EH1, EH2, MEFs p16 (WT) & their p16-specific knockout counterpart, Huh7 (hepatocarcinoma cell line) & HFSN1 (primary normal human skin fibroblast)	2	↑: Apoptosis; Bax; cleaved caspase-3; E2F1 ↓: NF-kB; Bcl-2 & Bcl-xL
Spallarossa et al. 2010[43]	Cord Blood (CB), EPCs)	0.1, 0.25, 0.5, 1.0	↑: Apoptosis; β-galactosidase; p16INK4A with perinuclear accumulation; activation of p38 & JNK; F-actin disorganization ↓: TRF2 protein; proliferation; cell viability
Sung et al. 2018[44]	VSMCs	0.5	↑: mTOR signalling; p70S6K; 4E-BP1; β-galactosidase; LC3 II; expression of p53/p21/p16
Yang et al. 2012[46]	Chronic myeloid leukemic cell line K-562	0.05	↑: β-galactosidase; miR-375; miR-652; miR-22; miR139-5p; ATG9B & ATG18

Table 3: Characteristics and outcomes from exercise studies having therapeutic effects against doxorubicin

Reference	Tissue/cell	Type of exercise	Effect of exercise on skeletal muscle
Bredahl et al. 2016[50]	Rat soleus and EDL muscle	Resistance and endurance training	Resistance training maintained maximal twitch force and maximal rate of force decline in the soleus; Endurance training reduced doxorubicin-induced fatigue in the soleus but not EDL
Bredahl et al. 2020[48]	Rat soleus and EDL muscle	Resistance training and creatine	Resistance training delayed doxorubicin-induced fatigue by 20 s in the soleus and 10 s in the EDL; Resistance training and Creatine combined, delayed doxorubicin-induced fatigue by 50 s in the soleus and 20 s in the EDL
De Lima et al. 2018[42]	Murine gastrocnemius	Aerobic exercise	↑: Maximal aerobic capacity; AMPKα phT172/total AMPkα expression ↓: Doxorubicin effect to reduce protein synthesis
Dickinson et al. 2017[51]	Rat soleus muscle	Endurance exercise	Prevents doxorubicin-induced: REDD1 mRNA; mTOR and 4E-BP1 phosphorylation reduction; LC3BII/I ratio reduction and MHC I fiber size loss
Huang et al. 2017[7]	Rat soleus muscle	Eccentric exercise	Prevents doxorubicin-induced: increased inflammation score; increased M1 macrophage ↑: M2 macrophage ($CD163^{+}$) ↓: Necrotic fibres; centronucleation; TNF-α mRNA
Quinn et al. 2017[47]	Rat soleus, EDL and diaphragm muscle	Endurance exercise	↑: Myf5 in soleus and diaphragm; MyoD & Mrf4 in soleus
Kavazis et al. 2014[35]	Rat heart and soleus muscles	Short-term endurance exercise	Prevents doxorubicin-induced: increases of FoxO1 and MuRF-1 in cardiac muscle; increases of FoxO3, MuRF-1 and BNIP3 in soleus muscle ↑: PGC-1α in heart and soleus muscle
Smuder et al. 2011[36]	Rat soleus muscle	Endurance exercise	Prevents doxorubicin-induced: damaged myofiber ultrastructure; cell apoptosis; Beclin-1; Atg12 mRNA & protein; Atg7 protein; LC3 mRNA; LC3II/LC3I ratio
Smuder et al. 2011[38]	Rat soleus muscle	Endurance exercise	Prevents doxorubicin-induced: protein carbonyls; 4-HNE; calpain-to-calpastatin ratio; calpain and caspase-3 activity; degradation of actin; proteolysis ↑: GPX1 protein; HSP72 protein
Guigni et al. 2019[52]	Murine C2C12 myotubes	Muscle contraction by electrical stimulation	Prevents doxorubicin-induced: myosin loss; increased Murf1; decreased mitochondrial & Akt and FoxO3a phosphorylation.
Yoon et al. 2019[41]	Murine C2C12 cells	AICAR (endurance exercise)	↑: Phosphorylation of AMPK; cell viability ↓: Cell death
Kwon 2020[53]	Murine soleus muscle	Endurance exercise	Prevents doxorubicin induced: irregular myofiber size; centronucleation dislocation; MHC type IIa isoform and type I composition reduction; Inhibition of the Z-line expression of α-ACTN protein; FOXO3α activation.

Table 4: Characteristics and outcomes from growth factors studies that induces myogenesis against doxorubicin myotoxicity

Reference	Tissue/cell type	Growth factor	Dosage (Administration)	Condition (Dosage)	Effect of growth factor
Chen et al. 2018[58]	Rat cardiomyocytes	MSCs-induced VEGF release	(pharmacological)	Doxorubicin (0.5 μM)	↑: Cell viability; proliferation ↓: p53; p16; telomere shortening; telomerase activity
Lawrence et al. 1986[21]	Rat cephalad and caudad chamber	TGF-β, EGF, PDGF	100 ng/ml (pharmacological)	Doxorubicin (8 mg/kg)	TGF-β accelerates wound healing; TGF-β, EGF & PDGF combined reverse completely the inhibition of wound repair induced by doxorubicin
Yao et al. 2015[57]	Murine C2C12, human embryonic kidney 293 cells and A549 lung adenocarcinoma epithelial cells	EGF	500 or 20 ng/ml (pharmacological)	Doxorubicin (0.3 μM)	↑: GATA4 expression; Cell cycle-associated protein cyclin D1; cell viability ↓: Dox-mediated growth arrest
Koleini et al. 2017[24]	Rat cardiomyocytes	FGF-2	10 ng/ml (pharmacological)	Doxorubicin (0.5 μM)	Prevents: mitochondrial permeability transition pores mPTP formation; downregulation of transcription factor EB and lysosomal associated membrane protein-1 (LAMP-1) and cell death caused by dox. ↑: ATP; Nrf-2 protein and mRNA; HO-1 mRNA and protein; p62/SQSTM1; (p-Ser2448)-mTORC1/ total mTORC1 ratio ↓: LDH activity; caspase-3; p53; Bnip-3 protein; ADP levels; ROS levels
Koleini et al. 2018[54]	Rat cardiomyocytes	non-mitogenic FGF-2	10 ng/ml (pharmacological)	Doxorubicin (0.5 μM)	Protects against Dox-induced: oxidative stress; upregulation of fragmented and non-fragmented oxidized phosphatidylcholine species ↑: P-ERK; P-p38; P-AKT ↓: Cardiomyocyte damage; cell death
Sontag et al. 2013[55]	Murine heart	FGF-2 and FGF-16	10 μg (pharmacological)	Doxorubicin (10 μM)	↓: deleterious effect of doxorubicin on left ventricular developed pressure
Wang et al. 2017[56]	Rat cardiomyocytes (vitro), mice heart (vivo)	FGF21	50 ng/ml, 100 μg/kg (pharmacological)	Doxorubicin (5 μg/m, 5 mg/kg)	↑: SIRT1) binding to liver kinase B1 (LKB1); AMPK activation ↓: TNF-α; IL6; ROS formation; apoptotic cells; Bax/Bcl-2 expression; LKB1 acetylation
Wang et al. 2018 [60]	Rat heart and cardiomyocytes	FGF-16	Gene therapy (AdV transfection)	Doxorubicin (1 μM)	↑: Resistance to DOX-induced cardiomyocyte damage ↓: Annexin-V+ cells; LDH activity

Table 4: Characteristics and outcomes from growth factors studies that induces myogenesis against doxorubicin myotoxicity

Reference	Tissue/cell type	Growth factor	Dosage (Administration)	Condition (Dosage)	Effect of growth factor
Räsänen et al. 2016[23]	Murine liver, heart, epididymal adipose tissue, endothelial cells, cardiac microvasculature	VEGF-B	Gene therapy (AdV transfection)	Doxorubicin (6 mg/kg)	Prevents decrease of: heart weight; cardiomyocyte size; left ventricle posterior wall; septum thickness; body mass; coronary capillary area; ERK1/2 phosphorylation by doxorubicin Prevents damage of: microvasculature cardiac from doxorubicin Protects from: apoptosis; endothelial dysfunction induced by doxorubicin ↑: Cytoskeleton biogenesis; angiogenesis; cell cycle-related transcripts; left ventricle mass systolic and diastolic volumes; mitochondrial DNA (mtDNA) content; ↓: DOX-induced DNA damage
Chen et al. 2010[59]	Rat cardiomyocytes	VEGF165	Gene therapy (AdV transfection)	Doxorubicin (2 μM)	↑: Bcl-2; Akt/nF-kB/Bcl-2 signaling pathway ↓: Caspase-3; apoptotic cells; FADD/caspase-8
Esaki et al. 2007[22]	Murine heart, cardiomyocytes	HGF	Gene therapy (AdV transfection)	Doxorubicin (15 mg/kg ip)	↑: Myocardial expression of GATA4; MHC; activation of ERK; c-Met/HGF receptor ↓: Left ventricular dilatation and dysfunction; cardiomyocyte atrophy/degeneration; myocardial fibrosis

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